State of the art of the environmental behaviour and removal techniques of the endocrine disruptor 3,4-dichloroaniline

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Abstract

In recent years, the presence of Endocrine Disrupting Chemicals (EDCs) in wastewater discharges from agricultural and industrial sources, [1] fresh- and estuarine-waters, as well as soils, has been reported in the literature. [2] Studies of adverse changes in wildlife, linked to environmental exposure to these substances, and the suggestion that humans could also be at similar risk of adverse health effects, [3-5] have raised concern for urgent action to understand and reduce such risks. 3,4-dichloroaniline (3,4-DCA) has been recognized as an EDC, with regards to endocrine disruption data for both wildlife populations and human health. [5]

3,4-DCA is present in the environment as a product of the biodegradation of phenylurea and phenylcarbamate pesticides; [6, 7] furthermore, it can be introduced from industrial and municipal wastewater that is insufficiently purified, or via accidental spills. [8-10] Increasing concentrations of 3,4-DCA in soil and water are the result of its high persistence and accumulation, as well as its low biodegradability. [11, 12] Hence, remediation techniques require in-depth study, especially when considering the low removal achieved by traditional activated sludge treatments, and the generation of carcinogenic trihalomethanes as a consequence of the chlorine oxidation methods

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frequently used in drinking water plants. [13] Fe^0/H_2O_2 systems, photodegradation using doped TiO_2, and the use of dielectric barrier discharge reactors, seem to be the most promising techniques for the removal of 3,4-DCA from water.

Keywords: 3,4-DCA; water; environment; pollution; adsorption; electrochemistry

Introduction

Global annual production of 3,4-dichloroaniline (3,4-DCA) was ~42-47 kt prior to 1986 [14] and, although recent years have seen a decrease in scale, EU production was still around 13.5-15.5 kt $annum^{-1}$ in the period 1996-1998. [15] 3,4-DCA, a derivative of aniline, is an intermediate in the chemical synthesis of 3,4-dichlorophenylisocyanate, to make phytosanitary products, such as propanil, linuron, diuron, and neburon, [15-17] used to treat crops including rice, potatoes, beans and tobacco. [18] It is also employed in the synthesis of azo dyes for polyester fabrics [17] and pharmaceuticals. [5] Although there is no exposure risk for vulnerable groups from direct use of 3,4-DCA, indirect contact is expected via food, primarily fruit and vegetables, [5] as a result of the hydrolysis and biological degradation of phenylurea, phenylcarbamates and acylchloroanilide pesticides present in soils, [19-27] by field waters and plant enzymes. [28] Furthermore, industrial wastewater [11] may contain 3,4-DCA, mainly from microbial conversion of 3,4-dichloro-1- nitrobenzol within water treatment plants. [5, 28]

In this manuscript, we summarize the current knowledge concerning the introduction, movement, and fate of 3,4-DCA in the environment. We discuss the state-of-the-art remediation technologies currently in use, as well as those under development, with reference to their effectiveness for pollutant removal from soil and water systems.

Toxicity

In humans, EDCs are known to affect male and female reproductive organs, thyroid metabolism, [29-31] breast development, cardiovascular and neuro-endocrinology, [32] causing obesity and

some cancers, including prostate cancer. [33] Little is known about the mechanisms of action of these substances, nor their physical and chemical diversity, hence, additional research into EDCs is required, especially on the cumulative impact of EDC mixtures, which may be additive or synergistic, [32, 34] even when individual chemicals are present below the threshold of detectable effects. [35] Consequently, the current lack of knowledge regarding exposure scenarios hinders the assessment of human health risk and impact. [36]

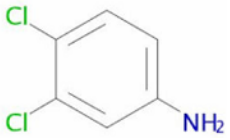
Chloroanilines can negatively affect soil microflora, and the presence of phenylamide herbicide degradation products has been shown to inhibit *Nitrosomonas*, hence, soil nitrification. [37] 3,4-DCA primarily acts by polar narcosis on aquatic organisms; [38, 39] fish and crustaceans are extremely sensitive with respect to water column exposure, while annelids are greatly affected by exposure to the sediment. [17] Tests have demonstrated the relatively low, acute toxicity of 3,4-DCA on marine and fresh water animals, as well as unicellular algae, but there are significant chronic effects [40, 41] with consequences for marine life reproduction, [4] including changes in secondary sex characteristics; [42] while rats have shown significant hormonal effects. [3] As a consequence of these impacts, Predicted No-Effect Concentrations (PNECs) have been proposed, which are 0.2 $\mu g\ L^{-1}$ for freshwater and seawater bodies, and 0.1 $mg\ kg^{-1}$ dry weight (0.04 $mg\ kg^{-1}$ wet weight) by mass [17] for sediments.

Environmental behaviour and fate

Chloroanilines are known to diffuse easily within the natural environment, [1, 14] and are very difficult to remediate, hence, their concentration in soils and waters is increasing due to their high persistence, accumulation, [11, 12] low biodegradation [43] and low leaching potentials. [44] By contrast, 3,4-DCA is barely measured in water bodies, [5] and it occurs in higher aqueous concentrations than the parent herbicide diuron. [45] It has been detected in European surface waters, [16, 46] and its migration is determined by transport and retention mechanisms. [47]

Hydrolysis of 3,4-DCA is not considered a mitigating mechanism due to aromatic stabilisation effects, [48] and, as a result of its relatively low Henry's constant (K_H), it is not expected to volatilise from water columns. [17, 48] Rather, as the physical data presented in Table 1 show, 3,4-DCA losses from natural waters occur via photochemical degradation, or adsorption on sediment and dissolved humic materials. [49] To this end, photo-transformation is the major degradation pathway of 3,4-DCA in environmental waters, [16] maximised at 300 nm, well within the solar spectrum observed at sea level. [4]

Table 1. Properties of 3,4-dichloroaniline

Property	**Value**	**Ref.**
Molecular formula	$C_6H_5Cl_2N$	-
Molecular structure		[15]
Appearance	Solid at 293 K	[15]
Molecular weight	162 g mol^{-1}	[15]
Molecular size	0.35 nm^2	[50]
Henry's constant	0.05 Pa m^3 mol^{-1}	[15]
Solubility in water	580 mg L^{-1} at 293 K	[15]
Octanol-water partition coefficient (log K_{ow})	2.7 (shaken flask method)	[5, 16]
Estimated surface water half life	18 days	[15]
Measured rate of loss from outdoor water systems	0.11 - 0.17 day^{-1}	[4]
	0.06 - 0.14 day^{-1}	[49]
Estimated atmospheric half life	9 hours	[15]
Estimated half-life in soil and sediment	470 - 1500 days	[51]

It is known that soil mobility and bioavailability of pollutants, and their degradation products, depend on a combination of adsorption and desorption by soil components. [47] The binding effect of soil generally increases with time, leading to a decrease in pollutant bioavailability and toxicity, due to meteoric leaching. [52] Hence, the majority of 3,4-DCA released into the environment accumulates, over time, on the organic fraction of sediments and soils. [15] On the other hand, sorption on dissolved macromolecules and colloidal particles promotes transport in subsurface environments. [53] When chloroanilines are released into the soil with herbicides, only a small portion of the former is mineralized, consequently, many chloroanilines persist for years, [54] often immobilized by interaction with humic substances, as mentioned above. [9, 20, 55, 56] Therefore, it is only a small fraction of liberated chloroanilines that undergo dimerization or polymerization, by microbial oxidases and peroxidases, forming stable azo compounds. [47, 57] Surface adsorption, due to van der Waals forces or electrostatic interactions, is often the initial phase of pollutant binding by soil, while stronger bonds may occur over time. [1] Consequently, fulvic and humic acids play a significant role in the binding of xenobiotics, such as chloroanilines, from aqueous media, via functional substituents, including hydroxyl, carboxylic acid, ketone, amino acid, saccharide and aminosaccharide groups. [58, 59] Aniline sorption to soil involves stronger interactions, starting with hydrophobic partitioning and cation exchange, before covalent bonding, due to the contribution of limited energy or availability of sorption sites, occurs. 3,4-DCA adsorbs onto sediment [49] and soil particles, building stable, most probably, covalent bonds with organic substances. [16, 17] Such initial reversible equilibrium, followed by a slower irreversible mechanism, is well described by a biphasic kinetic model. [60, 61] Similar information on the sorption mechanisms of 3,4-DCA is fundamental to the development of remediation strategies. In laboratory experiments, more than 70% of radio labelled 3,4-DCA was found, as stated above, to bind to sediment and suspended matter in a water column; [62] ~80% of radioactivity was removed from the water column within 8 days, and

~99% after 90 days, [63] suggesting an initially quick process, which then plateaus and requires significant time to reach its final equilibrium. As a consequence of the proposed interaction between 3,4-DCA and organic materials, the interactions between dissolved organic matter from soil, organic contaminants, and other soil components strongly affect the fate of 3,4-DCA in soil and water systems. [64-67] As a result, increasing soil organic carbon content, via organic matter amendments to a soil sample, which may also introduce dissolved organic matter, can potentially enhance sorption, and decrease leaching, of pollutants, [68, 69] including 3-4-DCA; [49] notably, similar results have been observed for organic matter amendment of inorganic soils. [8, 47]

A study into the interaction of various humic fractions and the herbicide diuron [70] showed that the main degradation product, 3,4-DCA, was irreversibly bound on humic acids within days of formation. As a result, the risk of pollutant leaching is expected to be low for soils with high humic or fulvic acid contents. The irreversible sorption observed in these systems was described using a Freundlich isotherm model:

$$C_s = K_f C_e^n \qquad (1)$$

where C_s is the concentration of 3,4-DCA sorbed ($\mu g\ g^{-1}$), C_e is the equilibrium solution concentration of 3,4-DCA ($\mu g\ mL^{-1}$) and K_f ($mL\ g^{-1}$) expresses the soil sorption capacity. The exponent n is related to the degree of isotherm nonlinearity, and provides an indication of the favourability of the sorption process. [71]

The results of Freundlich analysis indicated that humic fractions have a sorptive capacity ten times that of their fulvic counterparts, due to preferential reaction of the amino groups of 3,4-DCA with the carboxyl and carbonyl groups of soil humic fractions, leading to the formation of soil bound residues, [72, 73] as confirmed by high adsorption and small desorption rates constants. [49] Hence, the sorption of 3,4-DCA in soil can be described as a physicochemical process, in

which a fraction of the pollutant physically binds to organic and inorganic soil components, while another fraction strongly adsorbs on the organic component. [43] Similarly, soil samples, agitated in aqueous solutions of 3,4-DCA, have shown significant pollutant removal within 15 minutes of exposure and full equilibration, demonstrating up to 70% removal, after 50 hours. The authors of this previous study reported the presence of two associated processes, firstly a physical reaction, seemingly followed by chemical bond formation between 3,4-DCA and organic matter within the soil, giving biphasic kinetics with rate constants of 4.9 $hour^{-1}$, for physical accumulation, and 0.03 $hour^{-1}$, for chemisorption. [49] Similar kinetic performance has been observed for different agricultural soils and 3-4-DCA, [43] with sorption equilibrium reached within 48 hours regardless of initial pollutant concentration, up to 16.2 $\mu g\ mL^{-1}$. Again, the data was satisfactorily described by the Freundlich equation, and the amount sorbed was highest for the sandy clay loam soil used in the study (K_f = 52 mL g^{-1}), as a consequence of its higher organic matter content compared to the other soils studied, and the fact that it creates a slightly acidic pH within the sorption system. By contrast the lowest sorption capacities were obtained for calcareous silty clay soil and sand.

As initial adsorbate concentration increases, the availability of adsorption sites decreases, as confirmed by *n* values lower than 1; such sorption behaviour being expressed by L-type isotherms. [74] K_{oc} values, representing the sorption constant per gram of organic carbon in the soil sample studied, show significant probability for 3,4-DCA contamination of ground-water for soils with lower sorption potentials. [44] This is due to the strong relationship exhibited between the sorption capacity and organic matter content of soil samples, and the fact there is less impact with respect to clay content or a material's cation exchange capacity. It is also noteworthy that consideration of soil organic content alone is insufficient to determine expected sorption behaviour; for example, a lower level of diuron sorption is expected on clay-rich soils, most likely as a result of a reduction in available binding sites in humic substances, due to the

153 positive interactions between humic materials and clay. [70] Soil pH may be another factor that
154 impacts on sorption of 3,4-DCA, as demonstrated by a reduction in K_f values, by ~50%, after
155 liming of aqueous solutions. [43] Such a trend probably results from the presence of aniline
156 functionalities within the soils tested, which are normally protonated species under the usually
157 acidic conditions found in such media, becoming neutral species as pH increases. [61, 75, 76]
158 Samples of calcareous soil mixed with aqueous 3,4-DCA, and allowed to equilibrate fully [47]
159 showed high K_f values, which indicates that adsorption is concentration dependent, [77] thereby
160 validating the assumption that adsorption on such solid media occurs primarily via hydrophobic
161 interactions due to the neutralisation of aniline functional groups at high soil pH. [73, 78]
162 Additional confirmation is provided by consideration of the physical properties of 3,4-DCA; K_{ow}
163 is known to be high, [9, 79] its solubility on water is low, combined with a K_{oc} of 338.6 L kg^{-1}
164 ($K_{oc} = K_d/[\text{organic content}] \times 100$, where K_d is a measure of the distribution ratio of organic
165 molecules between the sorbed phase and solution), this all suggests that 3,4-DCA has a low
166 potential for groundwater contamination in calcareous soils with high organic matter content.

167 Dissolved organic matter has been proven to compete with organic pollutants for the sorption
168 sites available on soil surfaces, [80, 81] as well as in the building of stable bonds between pollutant
169 species and soil; [82] this competition enhances the apparent solubility of organic pollutants,
170 thereby reducing sorption and increasing their mobility. [67, 83] In contrast, dissolved organic
171 materials applied in soils may actually be adsorbed to soil surfaces, increasing sorption of
172 hydrophobic organic compounds, [84] especially when tannic acid is added. [8] Inclusion of
173 dissolved organic carbon extracts, derived from both a commercial peat and high-purity tannic
174 acid, to a soil sample showed significant impact on the sorption of 3,4-DCA. [47] The $K_{d,DOC}$
175 coefficient, defined as X_{doc}/C_{doc} (where X_{doc} is mg L^{-1} of dissolved organic carbon and C_{doc} is
176 the corresponding equilibrium concentration), is consistently higher than the K_d values obtained
177 for undoctored soil, thereby confirming the influence of dissolved organic carbon on 3,4-DCA

sorption. Further confirmation is provided by an increase in 3,4-DCA sorption in the presence of an environmental matrix, including inorganic ions and organic matter. [85] Sorption on sedimentary material, hosted in a sediment extract media, was higher than for pure or run-off waters, likely facilitated by previously sorbed dissolved organic content on the sediment surface, present as a result of physical interactions between the two species. [73]

Remediation technologies

Remediation technologies developed for the destruction of chloroanilines present in wastewaters can be classified as physicochemical, chemical (oxidation) or biological, and are discussed in detail below. In essence, physicochemical methods utilise either adsorption, ion exchange, electrolysis or photodegradation, chemical processes require a chemical reaction with a selected additive, while biological degradation involves the action of aerobic or anaerobic microorganisms.

Bioremediation

Bacteria

3,4-DCA is not readily biodegradable; [17] the process being particularly slow in aqueous media. Incubation of pond water, and pond water containing sewage sludge inoculum, in a darkened environment showed that, after a period of 2 weeks, 97% of 3,4-DCA was recovered from the former sample and marginally less (94%) was recovered for the sample containing sewage, indicating little biodegradation of the pollutant. [86] Similarly, no biodegradation was reported, over the same time period, for an OECD 301 C test [87] with activated sludge, [88] nor after 4 weeks for an OECD 301 D test on activated sludge, and less than 5% degradation was observed after 29 days for an OECD 303 A test, [87] again using activated sludge. [17] Researchers also observed no discernible removal of 3,4-DCA from contaminated North Sea

water samples, [89] while only primary degradation occurred after one month using river water as an inoculum. [90]

Usually, xenobiotics need to be in an aqueous phase in order to allow bacterial degradation to occur. The '*bioaccessible fraction*' of a pollutant is given by the sum of its concentration in pure water, known as the '*bioavailable fraction*', plus the '*potentially available fraction*', which is the material reversibly sorbed on any material surfaces. The addition of fulvic and humic acids to inoculated soils was seen to decrease the rate of diuron degradation, reducing bioavailability but not bioaccessibility, hence, lengthening the treatment time required; so it was only after 32 days that all diuron was degraded to 3,4-DCA. [70] Hence, bioaccessibility is a better indicator than bioavailability of the long-term influence of humic substances on diuron degradation. The mineralisation rate of 3,4-DCA in soils is low, and it decreases as pollutant concentration increases; [15] only 3.9-11.9% mineralisation of 1 mg kg^{-1} radio labelled 3,4- DCA was recorded after 16 weeks within various soil types. [51] Degradation of 50% of 3,4-DCA was observed in soil slurries with indigenous soil populations, and this was only marginally influenced by the addition of buffer, mineral salts and acetate. [91] In non-acclimated sediments, dechlorination of applied 3,4-DCA started after 20 days, with anaerobic conversion to 3-chloroaniline (44%) and 4-chloroaniline (33%) within two months; these metabolites were not further degraded. [92]

The microbial strains *Pseudomonas* acidovorans [93] and *Pseudomonas* diminuta [94] are able to use chlorinated anilines as a sole source of carbon and energy ; the latter also being capable of growing on 3,4-DCA. [95] Addition of *Pseudomonas* acidovorans to 3,4-DCA enriched soil slurries enhanced pollutant mineralization, leading to complete elimination of chloride after 10 days. [91] Up to 250 mg L^{-1} of the pollutant and its intermediates were anaerobically degraded, in under 7 days, by a strain of *Pseudomonas* fluorescens. Without added glucose and nitrogen

sources, degradation was slower, with 40% of toxicant removal in the first 15 days, at an initial concentration of 75 mg L^{-1}. [96] *Pseudomonas* diminuta was proven to dechlorinate up to 50 μg mL^{-1} of 3,4-DCA within its growth process, increasing the ratio of degradation in water samples from natural water reservoirs [97] and in fish ponds, where significant degradation of reversibly sorbed pollutant was observed within the first 12 days. It is also notable that an appreciable decrease in the irreversibly sorbed fraction occurred within the first 5 days. [98]

Microorganisms, from Cuban soils, were grown in two culture media, using 3,4-DCA, firstly as the sole source of carbon and secondarily as the sole source of carbon and nitrogen. [99] The pollutant was completely consumed within 3 weeks using *Pseudomonas*, *Arthrobacter*, *Aspergillus*, *Penicillium*, and *Fusarium*, isolated in the first medium, while *Bacillus*, *Arthrobacter*, *Cunninghamella*, *Trichoderma*, and *Fusarium* were isolated in the second system, demonstrating myriad bacterial growth from 3,4-DCA as a feedstock. The biodegradation pathway of 3,4-DCA, and other substituted anilines, involves conversion, by oxygenase, to the corresponding catechol, which is then metabolised via an ortho-cleavage pathway. [100] Two modes of dioxygenation have been determined, utilising degrading bacteria obtained by genetic exchange between two strains of *Pseudomonas*, and leading to the formation of 3- and 4-chlorocatechol from 3-chloroaniline. In contrast, only 4-chlorocatechol was generated from dioxygenation of 4-chloroaniline. [94] When bacterial strains of *Pseudomonas* acidovorans were used for the degradation of 3-chloroaniline and 4-choloranaline, the rate-limiting degradation steps were found to be the first attack of the substrate, and conversion to chlorocatechols. [93]

Degradation of 3,4-DCA by *Pseudomonas* sp. showed that catechol 2,3-dioxygenase is integral to process efficiency; [95] activity toward 4-methylcatechol, 3-methylcatechol and 4-chlorocatechol was found to be 60, 27 and 13%, respectively, of the activity toward catechol 2,3-dioxygenase. Further tests confirmed catechol 2,3-dioxygenase activity using a strain of

Pseudomonas fluorescens; the presence of 3-chloro-4-hydroxyaniline as a metabolite suggesting a pathway that includes dehalogenation and hydroxylation of the aromatic ring, followed by ring cleavage, by catechol 2,3-dioxygenase. [96]

Recent work has shown *Micrococcus* sp. to degrade 96% of diuron within 30 hours of incubation, at a concentration of 250 ppm, and with the addition of non-ionic detergent (0.01%). [101] The authors proposed a mechanism whereby a methyl group is removed, followed by a hydrolysis step, leading to the accumulation of 3,4-DCA, which undergoes conversion to 4,5-dichlorobenzene-1,2-diol, and further intermediates, within 24 hours of test commencement (Fig. 1). Diuron mineralization has also been confirmed by the metabolic cooperation of *Arthrobacter* sp. and *Achromobacter* sp., with CO_2 as the only final product. [102]

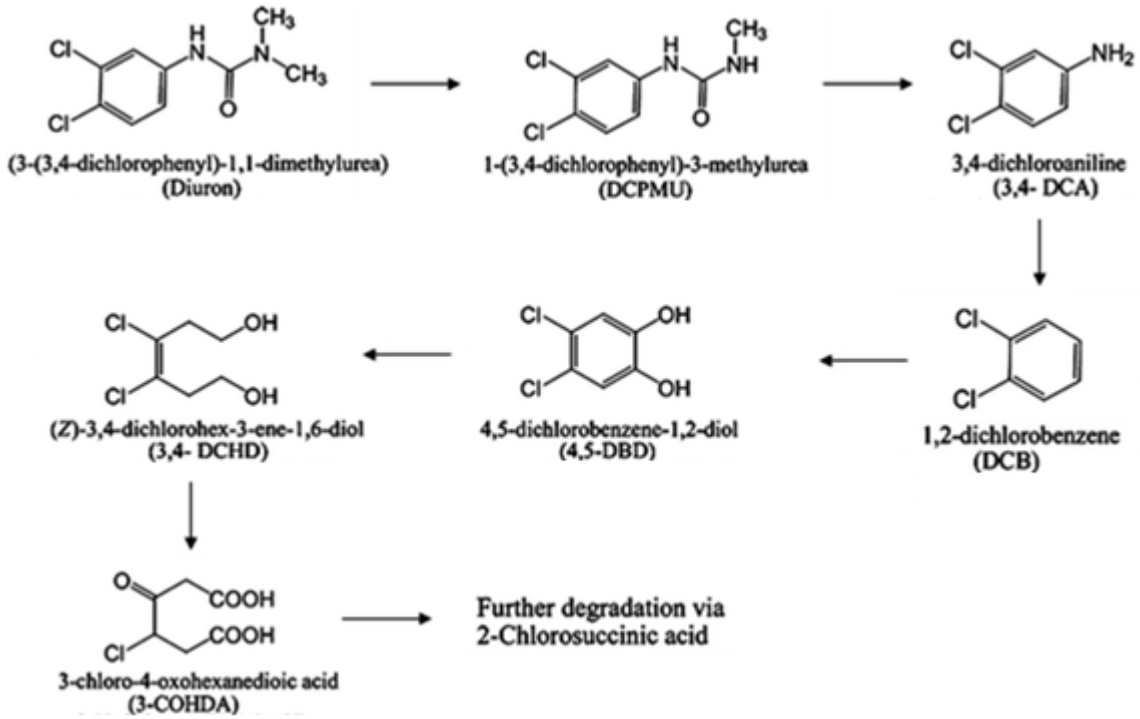


Figure 1. Degradation pathway of diuron by *Micrococcus* sp, confirmed by FTIR spectra and HPLC [101]

Strains of *Aquaspirillum* itersonii, *Aquaspirillum* sp. and *Paracoccus* denitrificans were shown to successfully use 3,4-DCA as the only source of carbon and nitrogen for growth; [103] the latter was able to metabolize the pollutant at concentrations up to 150 mg L^{-1}, through oxidation to *o*-diphenol, intradiol cleavage of 4,5- dichloropyrocatechol, and further stages of preparatory metabolism associated with dehalogenation. A study of microorganisms isolated from Cuban soils, treated with propanide, showed a *Paracoccus* denitrificans strain to be most efficacious for 3,4-DCA [50] degradation. The cleavage of the aromatic ring via the ortho- or meta-pathways, following formation of 4,5- dichloropyrocatechol, allows full decomposition. Successful adsorptive bioremediation was demonstrated with the introduction of activated carbons and bacteria to polluted soils. [104] Activated carbon acts as buffer, which keeps the pollutant concentration in the soil solution below the toxicity threshold for the bacteria, as shown by a study of three types of activated carbon saturated with 3,4-DCA and placed in a mineral medium with a Paracoccus denitrificans strain. [50] The pollutant was reversibly sorbed, therefore available, at a limited concentration, for the bacteria population to process. Varied degradation rates, from 2 to 10 weeks, suggest that facile desorption and more rapid decomposition are linked to a reduced micropore volume. This can be ascribed to the fact that *Paracoccus* denitrificans is only able to penetrate into the macroporous structure, due to size exclusion effects, thus pollutant desorption from the smaller carbon pores is necessary for the bacteria to process any 3,4-DCA sorbed therein; this bacterium seems able to accelerate desorption by acidifying the medium or via excretion of surface-active substances.

Biological studies have also shown microbial consortia to be successful in the removal of propanil and 3,4-DCA from repeated batch suspended cell cultures, [105] as well as in biofilm reactors for agricultural wastewater treatment. [106] In the latter case, the porous volcanic stone tezontle was used as a support for a biofilm in a continuous process able to degrade propanil and metabolic intermediates at rates of up to 24.9 mg L^{-1} h^{-1}, without the need for co-substrates.

287 *Pseudomonas* sp., *Acinetobacter* calcoaceticus, *Rhodococcus* sp., *Xanthomonas* sp. and *Kokuria*
288 strains can also grow individually in 3,4-DCA, while other strains found in the biofilm, not able
289 to degrade propanil metabolites directly, are probably involved in the metabolization of
290 herbicide adjuvants or in the maintenance of biofilm integrity. Resultantly, the removal of
291 chloroanilines from sewage treatment plant streams could be improved by promoting the growth
292 of indigenous bacterial communities, and through the introduction of adapted laboratory strains.
293 The addition of readily degradable aniline and non-toxic haloaromatics may, respectively,
294 improve the breakdown of chloroanilines and the chlorocatechol potential. [94]

295 *Uptake by fungi and cultivated plants*

296 When free chloroanilines are released as herbicide metabolites, they can be incorporated in the
297 plant's 'insoluble' residue fraction; degradation experiments have proposed lignin as a primary
298 binding site. [107, 108] Immersion of the bivalve *Corbicula fluminea* in cages both upstream and
299 downstream of conventional rice fields, in the region of Camargue, [19] showed the concentration
300 of 3,4-DCA measured downstream of the rice plantations to be approximately half that in the
301 *Corbicula* caged upstream, suggesting a potential, partial bioaccumulation of 3,4-DCA in rice
302 plants. Tomato plants, oat, barley and wheat, grown in nutrient solutions with 4-chloroanaline
303 and 3,4-DCA showed that 90-95% of the chloroanilines incorporated were found in the roots,
304 with uptake proportional to the amount of chemical applied. In contrast, distribution of the same
305 chemicals in carrots was approximately equally divided between the roots and the upper part of
306 the vegetables. [109] These results suggest a potential risk of chronic toxicity due to the
307 assumption of 3,4-DCA contained in certain foods.

308 Some lignin degraders are able to metabolize chloroanilines and their lignin conjugates;
309 experiments have shown that chloroanilines appear to be bioavailable to the white rot fungus
310 *Phanerochaete chrysosporium* once they were mineralized as lignin. [110] More than 50% of

311 available [ring-U- 14C] -3,4-DCA was shown to be mineralized after 33 days of sample
312 incubation and free 3,4-DCA was deemed a superior substrate for mineralization than free 4-
313 chloroanaline. Different metabolites were formed, but chloroanilines were not detected, neither
314 were their azo or azoxy derivatives. Hence, lignin incorporation and fungal oxidation can lead
315 to the complete removal of 3,4-DCA from the environment; however, fungi is also known to
316 adsorb less chloro-substituted anilines per biomass unit than bacteria, [111] and the degradation
317 pathway of white-rot fungi could lead to the formation of toxic tetrachloroazobenzenes. [112]

318 ***Adsorption and ligand exchange***

319 Adsorption technologies offer effective removal of many organic pollutants from aqueous
320 media, [113] and various adsorbents have been studied for removal of chloroanilines from
321 wastewaters. Batch adsorption experiments, conducted with an acid activated halloysite, using
322 aqueous solutions at pH ~5, [12] gave experimental data that followed a pseudo-second order
323 kinetic model: [114]

$$\frac{t}{q_t} = \frac{1}{(k_2 q_e^2)} + \frac{1}{q_e t} \qquad (2)$$

324 where: q_t is the amount of chloroaniline adsorbed (mg g^{-1}) at time t (s), k_2 is the rate constant of
325 pseudo-second order adsorption (g mg^{-1} min^{-1}) and q_e is the amount of chloroaniline adsorbed at
326 equilibrium (mg g^{-1}). A 'Weber–Morris' plot of q_t versus $t^{0.5}$ confirmed that chloroaniline
327 removal occurred, first by fast diffusion of 3,4-DCA to the surface of the clay mineral, over the
328 first 180 minutes, before continuing as slower interparticle diffusion. The adsorption capacity of
329 halloysite was found to be lower for 3,4-DCA than for 3-dichloroaniline and 4-dichloroanaline.

330 Equilibrium isotherm data was effectively described by the semi-empirical Langmuir equation:

$$\frac{C_e}{q_e} = \frac{1}{(K_L q_m)} + \frac{C_e}{q_m} \qquad (3)$$

where: q_m is the monolayer adsorption capacity (mg g^{-1}), q_e is the sorption uptake at equilibrium (mg g^{-1}), C_e is the equilibrium solution concentration (mg L^{-1}) and K_L is a coefficient related to the affinity between the adsorbent and the adsorbate (L g^{-1}). Similar adsorption experiments have been performed with kaolinite (KGa-1) and montmorillonite (SWy-1), using standard solutions of 3,4-DCA and other chloroanilines at pH ~5 and ~9, respectively; [1, 75] the specific pH conditions were chosen so as to produce neutral organic pollutant species. Kinetic evaluation indicated an initial, rapid surge in chloroaniline removal, with equilibrium achieved in under 4 days. Langmuir and Freundlich equations both adequately described the data; [75] however, the Langmuir plot showed a marginally better fit for montmorillonite, hence adsorption on this clay is likely to decrease as surface sorption sites are saturated. The chloroanilines studied would be mainly sorbed on the mineral surface of kaolinite, while the structure of montmorillonite allows swelling via sorption in the interlayer, [115] as confirmed by studying the dehydrated clay; [1] post heat-treatment, the distance between equivalent atomic planes (d_{001}) collapsed from 11 to 9.7 Å. Further evidence was provided by X-ray spectra of the montmorillonite/3,4-DCA system. This contrast in sorption mechanism is reflected in desorption measurements, where kaolinite was shown to retain the pollutant, while montmorillonite showed a higher level of desorption when reversing the sorption process at an earlier point in the isotherm. [75]

While clay materials offer surface sites for adsorption, the main surface area of another well-known family of sorbents, i.e. activated carbons, is ascribed to microporous character; surface hydrophobicity determines the sorptive capacity of many organic molecules, which have molecular sizes small enough to penetrate into the micropores. Highly microporous activated carbons, obtained from coal and peat, were confirmed as better sorbents than mesoporous carbons, obtained from raw plant materials. Increased iron content and other ash elements may

positively influence the maximum uptake, by enhancing chemisorption of active organic compounds, such as 3,4-DCA. [50]

Studies of aqueous solutions of various pesticides agitated with 10 mg L^{-1} of powdered activated carbon (surface area ~1000 m^2 g^{-1}; particle size 40 μm) for 5 minutes, showed 70% removal efficiency for 3,4-DCA (initial concentration: 658 ng L^{-1}), while complete removal was achieved via preoxidation with ozone. [103] Fitting of room temperature sorption isotherms, obtained using suspensions of activated carbon in aqueous solutions of 3,4-DCA, [50] using the Langmuir isotherm model (Equation 3), showed monolayer filling of the sorptive surface with 'L-type' isotherms, indicating strong interactions between the adsorbate and adsorbent. [116]

Sugar beet pulp, corncob, corncob char, perlite, vermiculite and sand have recently been studied for sorption of 3,4-DCA from aqueous solutions at pH 4.8. [85] These sorbents may be used in field conditions, so it is essential to understand their sorption behaviour in different matrices. However, liquid matrices containing cations and organic matter, except for corncob, showed no significant differences in maximum uptake of 3,4-DCA from pure water and run-off waters. The mechanism of sorption was satisfactorily described by the Freundlich isotherm model, with the highest sorption uptakes obtained at 99% removal from water for corncob char, and, 86% removal for sand. Of the sorbents studied, it is also known that vermiculite has a good resistance to mechanical abrasion. [117] Further investigations would be required to verify the adsorption capacity of low cost materials in field conditions, but it is encouraging that sorbents such as sand could present appreciable capacities capable of removing > 50% of 3,4-DCA, even from sediment extract media (Table 2).

Table 2. Surface area, pore size and adsorption data of different material tested for the removal of 3,4-DCA from water. The parameters q_m and b refer to the Langmuir model (Equation 3)

Adsorbent	Surface area ($m^2 g^{-1}$)	Pore volume ($cm^3 g^{-1}$)	q_m (mg g^{-1})	b (mL mg^{-1})	Equilibrium time (h)	Stirring method
Halloysite [12]	76.6	0.039	0.078	2.726	> 3	Rotary stirrer
Kaolinite [75]	-	-	0.311	9	> 96	Electromagnetic
Montmorillonite [75]	-	-	0.077	23	> 96	Electromagnetic
Activated carbon AG [50]	963	0.55	583	-	48	-
Activated carbon SKT [50]	1028	0.53	480	-	0.5	-
Activated carbon RS [50]	410	0.5	364	-	0.5	-

Within ligand exchange processes, polymeric chelating resins are able to selectively remove target contaminants; however, eluate recycle, regeneration of depleted adsorbent, and the high cost of transition metals, used as ligand complexing ions, are still significant obstacles towards commercial application of such processes in wastewater treatment. Currently, there are no studies on the application of ligand exchange processes for 3,4-DCA removal, but this would seem possible in light of the recovery of aromatic amines from water, at low concentration, as demonstrated for chelating resin-bound cobalt ions. [118] A mini-column apparatus with Co(II)-CDAE-sporopollenin resin was also tested for adsorption of chlorinated anilines, found to be described by a Langmuir model for 2-chloroaniline, 4-chloroaniline and 2,5-dichloroaniline. [6] The study showed similar values for the maximum adsorption capacity (q_m) for binding of 2-

chloroanilines and 4-chloroanilines, which were consistently lower than those for 3-dichloroaniline and 2,5-dichloroaniline onto the Co^{2+} matrix, suggesting that both electrical forces and steric hindrance are involved in the sorption process. This conclusion is supported by consideration of the inductive effects of *ortho*-Cl and *para*-Cl atoms, as well as the nature of these ligands, which contain charged groups and may offer steric hindrance. Moreover, steric hindrance around the amino nitrogen weakens binding to metal ions, causing faster migration of the aforementioned pollutants. [119] The adsorption of 3-chloroaniline was better represented by a Freundlich model, possibly as a result of a more complex type of binding than the independent and univalent binding described by a Langmuir model.

Chlorination, ozonisation, chemical precipitation and Fe^0/H_2O_2 systems

A common sequence of operations adopted in many drinking water plants is that of (i) preoxidation, (ii) adsorption, and (iii) coagulation. Preoxidation of an aqueous sample with a concentration of 658 ng L^{-1} of 3,4-DCA, performed using sodium hypochlorite, demonstrated 100% pollutant removal; [13] however, such treatments have an associated risk of carcinogenic trihalomethanes by-product formation. Hence, researchers have considered other oxidative species and ozonolysis of a sample, again with a concentration of 658 ng L^{-1} of 3,4-DCA, showed 85% pollutant removal; however, the subsequent coagulation and flocculation steps were found to be ineffective, while further adsorption treatment, with activated carbons, led to complete pollutant removal. [13]

The oxidizing potential of Fe^0 towards different organic compounds is well known; [120] Fe^0/ H_2O_2 systems can be used to reduce levels of diuron, and other pesticides, in polluted environments, as well as agricultural waste. Fe^0 promotes the reduction of H_2O_2 to hydroxyl radicals, generating Fe^{2+}, which, in turn, also produces hydroxyl radicals via further H_2O_2 reduction. A 10 mg L^{-1} diuron solution, also containing 2 mmol L^{-1} of H_2O_2 and H_3PO_4, was

412 flowed through a glass tube packed with 2 g of iron wool, and showed that pH strongly affected
413 the degradation process, allowing process optimisation. At pH 2.5 more than 99.9% of the
414 pesticide was removed after ten minutes, with only 1 μg L^{-1} of 3,4-DCA found remaining in the
415 effluent. [121]

416 ***Electrochemical and electrohydraulic methods***

417 An electrohydraulic discharge (EHD) method for the oxidative degradation of 3,4-DCA was
418 tested by exposing wastewaters to pulsed electrical discharges generated via submerged
419 electrodes. [122] UV radiation is produced by a plasma channel created by EHD, thereby,
420 generating a shockwave as it expands against the water. The degradation rate is expressed by:

$$\frac{dC}{dN} = -k_1 C_i - k_0 \qquad (4)$$

421 where d*C*/d*N* is the change in concentration per discharge, C_i is the initial substrate
422 concentration, k_0 is the zero-order term (an expression of direct photolysis) and k_1 is the first-
423 order term related to oxidation in the plasma channel region.

424 As part of an Advanced Oxidation Process (AOP), photocatalysis can be employed (i) for water
425 treatment in slurry reactors, where an additional step is required for the separation of any
426 suspended catalysts, or (ii) into reactors, where the catalysts are immobilized on adsorbents or
427 on membranes. Using sols of vanadium pentoxide and cerium oxide, added to a titanium
428 dioxide sol, allowed preparation of Ti–V and Ti–Ce catalysts, respectively; 0.1 g L^{-1} of each
429 powdered catalyst was added to agitated aqueous solutions of 3,4-DCA, irradiated in an annular
430 reactor at 140 mW cm^{-2}, and the Ti–V catalyst gave a higher degradation than Ti-Ce, due to its
431 band gap energy (which is more towards the visible region) and smaller particle size. The
432 kinetic plot suggested bi-phasic kinetics, with a sharp increase in rate after 45 minutes; further

bench scale reactor experiments showed 85% degradation in 106 minutes [11] and Figure 2 shows the intermediate species formed.

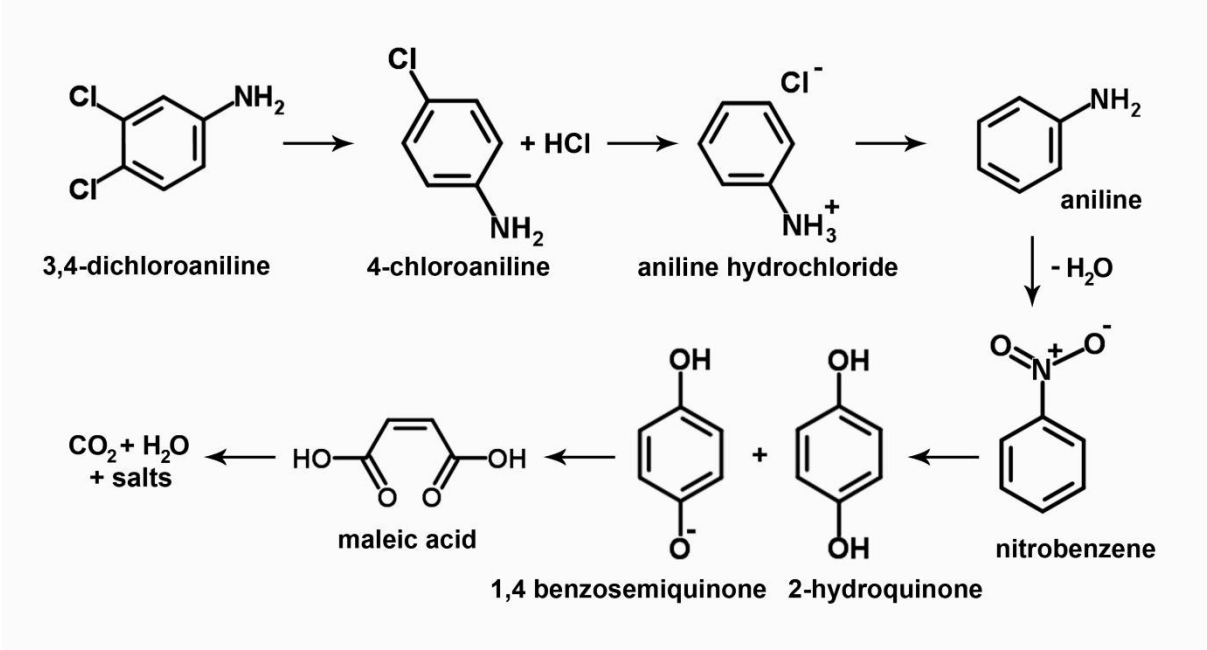


Figure 2. Intermediates formed during photo-degradation of 3,4-DCA using Ti-V (sol) catalyst, confirmed by HPLC and GC-MS analysis [11]

Biphasic kinetics were also observed in the photocatalytic degradation of 3,4-DCA using Ti–N and Ti-S catalysts; [28] the former showing a higher surface area, and lower particle size and band-gap energy. Higher specific surface area increases the degree of contact of the pollutant with the catalyst surface, while smaller particle size means a reduced degradation time, due to shorter distances between the charge carrier and the surface, where the reaction occurs. 3,4-DCA was fully degraded using a Ti–N catalyst in 120 minutes with optimal degradation obtained at pH = 6. At higher catalyst dosages (> 0.1 g L^{-1}) the reaction rate starts to decrease, due to the deactivation of molecules that collide with ground state molecules, and subsequent agglomeration of catalyst particles. Meanwhile, at 3,4-DCA concentrations > 10 mg L^{-1}, the degradation efficiency starts to decrease, as the number of collisions between the 3,4-DCA

molecules increases, while there is a reduction in collisions between pollutant molecules and •OH radicals. [123]

Degradation of aqueous 3,4-DCA was recently performed using a dielectric barrier discharge (DBD) plasma reactor; [124] where generated ozone interacts with the pollutant directly or by the generation of hydroxyl radicals, which results in a faster rate of reaction than for molecular ozone alone. [125] The process involves the flow of a water film through the discharge zone created by two aluminium electrodes, and the degradation process is well described by pseudo-first order kinetics, with higher efficiencies reached either under acidic conditions, increasing the input power or by adding Fe^{2+} or Fe^{3+}. The main pathways involved were deamination, hydroxylation, dechlorination, and hydroxylation and oxidation, followed by the generation of organic acids, via aromatic ring opening. Mineralization into CO_2 and H_2O was only partially achieved, as confirmed by the lower rate of removal for total organic carbon than for DCA, and by degradation intermediates identified using GC-MS analysis. Solution pH decreased during DCA degradation, as observed in previous work, [124] where aqueous 3,4-DCA was degraded by a wire-cylinder DBD reactor, with an efficiency that was observed to increase when the input power was increased to 90 W, but decreased at powers above that; aqueous ozone concentration was seen to follow the same trend. It was also noted that, similar to other systems, the process was pH dependent with a lower degradation rate obtained under neutral conditions than at acidic or basic levels.

Conclusions

Myriad technologies have been developed for the degradation of pesticides; however, more attention has to be mainly focussed on the fate of metabolites. 3,4-DCA is a degradation product generally more toxic than its parent substances; if it is covalently bound to humic

substances within soil, the risk of groundwater contamination via leaching is low, but it also has an extremely low rate of mineralization, [70] making it a significant environmental issue.

Lignin incorporation and fungal oxidation are able to effect complete removal of 3,4-DCA from the environment, [110] while the use of bacteria and porous materials can be successful in bioreactors, as well as for in-situ bioremediation techniques. Bioremediation has been successfully demonstrated, introducing activated carbons and bacteria to polluted soils, [50] and the biological removal of 3,4-DCA from sewage can be enhanced by the growth of indigenous communities, and through the introduction of adapted laboratory strains; [94] however, the technical and economic feasibility of such processes also need to be considered. Further investigations are required to confirm the adsorption capacity of promising low cost materials such as activated carbons, corncob char and sand, especially within field conditions. [85] The possibility of recovering aromatic amines from low concentration water streams has been already demonstrated using chelating resin-bound cobalt ions, [118] as well as for Co(II)-CDAE-sporopollenin resin, [6] but there have been no specific studies related to the application of such technologies to 3,4-DCA removal.

Fe^0/H_2O_2 systems could be developed for the degradation of 3,4-DCA in agricultural soils and waste, [121] while photodegradation using doped TiO_2 has been successfully performed, and degradation kinetics are available for the scale up of reactors. [11] Low toxicity and cost, combined with good chemical stability and high natural abundance [126] make this catalyst potentially suitable for the environmentally friendly removal of 3,4-DCA from water. Effective degradation is also observed for the use of dielectric barrier discharge reactors, but further studies are required to reach complete mineralization of 3,4-DCA. [124]

Hence, there is significant scope for the application of existing technologies to the issue of 3,4-DCA removal from aqueous streams, some of which have timely potential for implementation;

however, there is a need for more data to be acquired to permit economic and environmental impact of these proposed systems to be fully addressed.

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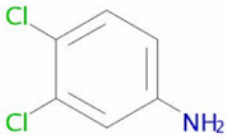
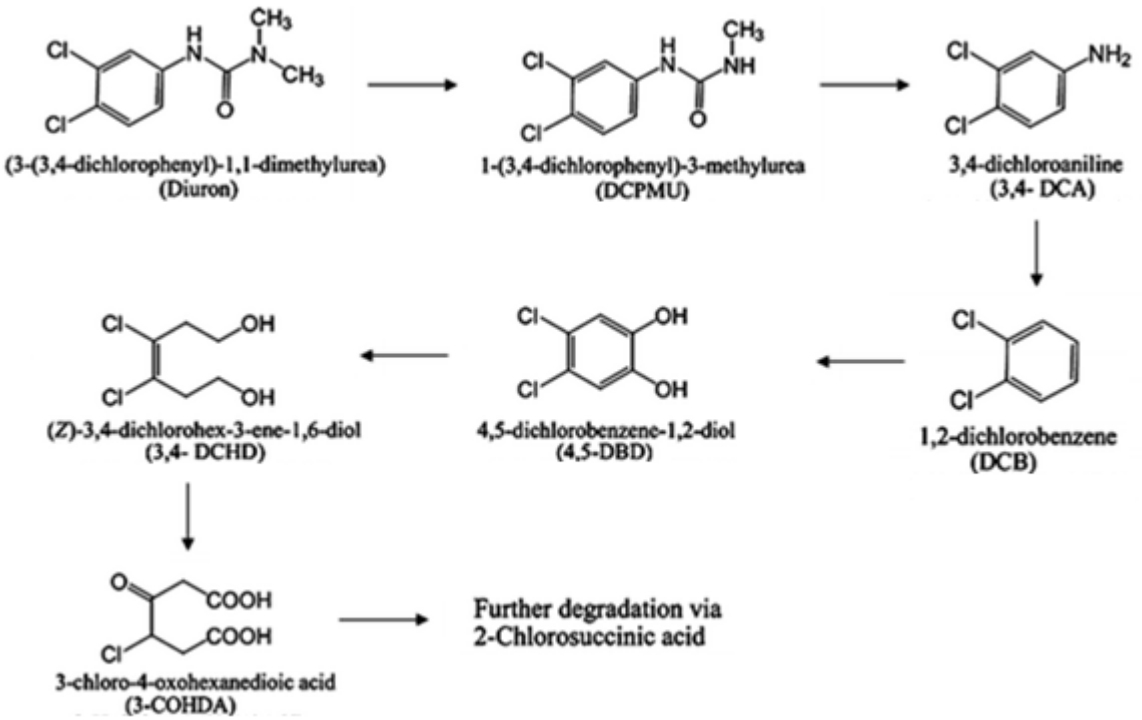
847 **Figures and Tables**

848 Figures and tables are listed below.

849 **Table 1.** Properties of 3,4-dichloroaniline

Property	**Value**	**Ref.**
Molecular formula	$C_6H_5Cl_2N$	-
Molecular structure		[15]
Appearance	Solid at 293 K	[15]
Molecular weight	162 g mol^{-1}	[15]
Molecular size	0.35 nm^2	[50]
Henry's constant	0.05 Pa m^3 mol^{-1}	[15]
Solubility in water	580 mg L^{-1} at 293 K	[15]
Octanol-water partition coefficient (log K_{ow})	2.7 (shaken flask method)	[5, 16]
Estimated surface water half life	18 days	[15]
Measured rate of loss from outdoor water systems	0.11 - 0.17 day^{-1}	[4]
	0.06 - 0.14 day^{-1}	[49]
Estimated atmospheric half life	9 hours	[15]
Estimated half-life in soil and sediment	470 - 1500 days	[51]

850



851

852 **Figure 1.** Degradation pathway of diuron by *Micrococcus* sp, confirmed by FTIR spectra and
853 HPLC [101]

854 **Table 2.** Surface area, pore size and adsorption data of different material tested for the removal
855 of 3,4-DCA from water. The parameters q_m and b refer to the Langmuir model (Equation 3)

Adsorbent	Surface area ($m^2\ g^{-1}$)	Pore volume ($cm^3\ g^{-1}$)	q_m (mg g^{-1})	b (mL mg^{-1})	Equilibrium time (h)	Stirring method
Halloysite [12]	76.6	0.039	0.078	2.726	> 3	Rotary stirrer
Kaolinite [75]	-	-	0.311	9	> 96	Electromagnetic
Montmorillonite [75]	-	-	0.077	23	> 96	Electromagnetic
Activated	963	0.55	583	-	48	-

carbon AG [50]						
Activated carbon SKT [50]	1028	0.53	480	-	0.5	-
Activated carbon RS [50]	410	0.5	364	-	0.5	-

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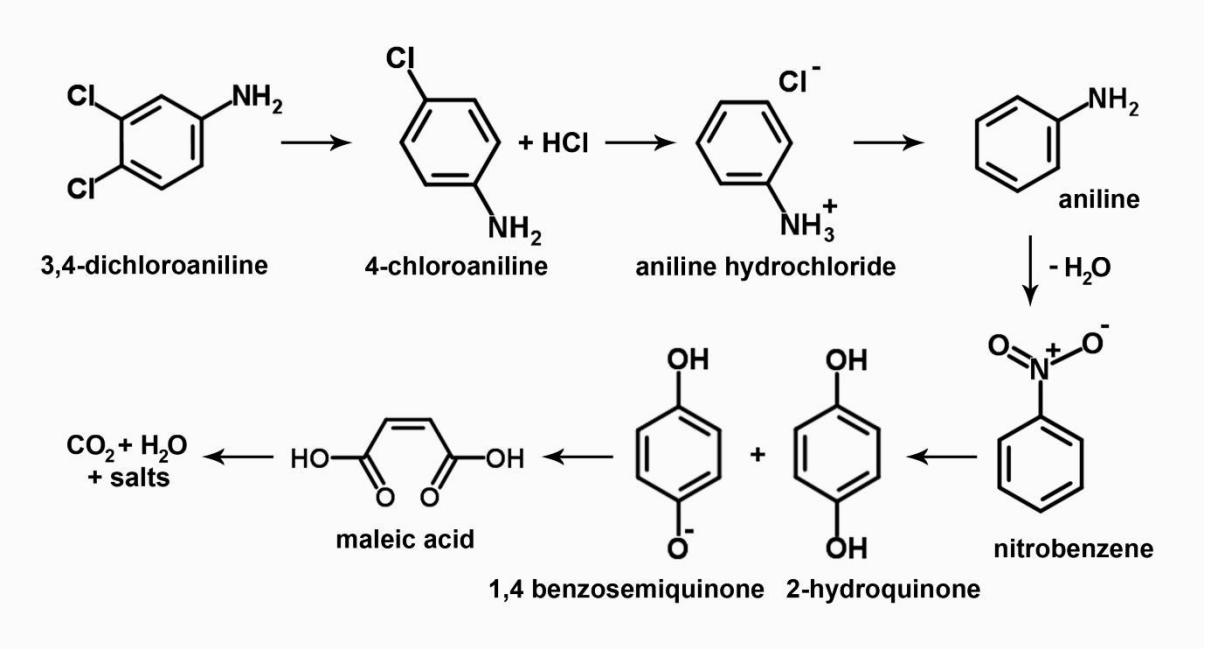

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859 **Figure 2.** Intermediates formed during photo-degradation of 3,4-DCA using Ti-V (sol) catalyst,

860 confirmed by HPLC and GC-MS analysis [11]

861